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The Toxicology of Carbon Tetrachloride

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Abbreviations:

Cyt,	cytochrome
CYP,	cytochromes P450
ER,	endoplasmic reticulum
Fe·S,	iron-sulpur protein
FP,	flavoproprotein
GS,	glutamine synthase
MDA,	malondialdehyde
NADH,	nicotinamide adenine dinucleotide (reduced)
NADPH,	nicotinamide adenine dinucleotide phosphate (reduced)
Q,	ubiquinone
Pco,	protein carbonyl

Table of contents

Abstract

Carbon tetrachloride (CCl_4) has been known as a hepatotoxin for a long time. It can induce both acute and chronic liver injury. Also, it is a carcinogen and can induce chromosome deletion. General believing, CCl_4 hepatotoxicity mainly depends on the reductive bio-activation to trichloromethy free radical ($CCl_3^{\bullet}$) by cytochromes P450. The mitochondria election-transport chain was also suggested for activation of CCl_4. $CCl_3^{\bullet}$ itself is highly toxic and may form many additional reactive intermediates *in vivo*. These free radical and reactive intermediates can cause lipid and protein peroxidation and are responsible for cellular damage caused by CCl_4 to a large extend. Recently, it becomes clear that secondary mechanisms are also evoked by initial events of CCl_4 metabolism. Among them, the disruption of calcium homeostasis is particular important.

1.Introduction

Carbon tetrachloride (CCl_4) is widely used to treat animals as a liver injury model because damage by CCl_4 is regarded as the analogue of liver damage caused by a variety of hepatotoxins in humans. It is generally accepted that the hepatotoxicity of CCl_4 results from the metabolism of CCl_4 to the trichloromethyl free radical ($CCl_3^{\bullet}$) by the NADPH–cytochrome P450 system, transferring an electron from NADPH to CCl_4 [1-2,9-12]. This free radical and related reactive species may cause cellular damage by initiating lipid and protein peroxidation, covalently binding to protein, causing a rise in intracellular Ca^{2+}, depleting GSH, or releasing iron, ultimately leading to cell death [1-4]. The hepatotoxic action of CCl_4 is known to be dependent on the cosubstrate NADPH because the primary metabolism of CCl_4 to $CCl_3^{\bullet}$ was formed in conjunction with the NADPH–cytochrome P450 electron-transport chain in the liver endoplasmic reticulum [1]. Cytochrome P450 transferred the electron from NADPH to CCl_4, causing CCl_4 to be reduced to $CCl_3^{\bullet}$ and Cl^- [7]. Rapid, extensive lipid peroxidation of the membrane lipids has been proposed as the basis of CCl_4 hepatocellular toxicity [1,7,10]. Various free radical metabolites (such as $CCl_3^{\bullet}$ and $CCl_3OO^{\bullet}$) were observed to generate after the treatment of CCl_4 [1,7,10].

2.The cytochromes P450 (CYP) and CCl_4 activation by CYP

The cytochromes P450 (CYP) constitute a superfamily of heme–thiolate enzymes, of which over 1200 individual members are known, and are present in species from all five biological kingdoms [2]. Cytochromes P450 catalyze the oxidative metabolism of a large number and variety of chemicals, both endogenous and exogenous [1-3]. The oxidative metabolism catalyze by CYP has a common unifying feature: the formation of new covalent bonds in the substate molecular with the incorporation of one atom of oxygen, which require NADPH and molecular oxygen [11]. In

addition, these enzymes are held responsibility for the reductive metabolism of several classes of compounds with a high oxidation status, including carbon tetrachloride [11]. These processes require selected substrates with highly oxidized functional groups and presumed to proceed by competition of the substrate with the oxygen for the elections supplied by NADPH [11]. That is, the substrate is thought to act as a replacement, not for oxidizable substrate, but for oxygen in the acceptance of elections, consequently, these reductive reactions often require anaerobic conditions, or at least low oxygen tension [11]. But the present of O_2 can amplify the cellular damage caused by the $CCl_3^{\bullet}$ —— the CCl_4 reduction product. Specially, O_2 is required for lipid peroxidation [9]. By the way, the cytochrome P450 may directly attacked by $CCl_3^{\bullet}$ [9]. These reactions are showed below [9,11].

$CCl_4 \xrightarrow{CYP,\ NADPH} CCl_4^{-} \rightarrow CCl_3^{\bullet} + Cl^{-}$ (k is not found) (1)

$CCl_3^{\bullet} + O_2 \rightarrow Cl_3C\text{-}OO^{\bullet -}$ ($k = 3.3 \times 10^9\ M^{-1}S^{-1}$) (2)

$Cl_3C\text{-}OO^{\bullet -} \rightarrow Cl_3C\text{-}OOH$ (k is not found) (3)

$Cl_3C\text{-}OO^{\bullet -} \rightarrow Cl_3C\text{-}OOH$ (k is not found) (4)

$Cl_3C\text{-}OOH \rightarrow Cl_3C\text{-}OH$ (k is not found) (5)

$Cl_3C\text{-}OH \rightarrow Cl_2CO$ (k is not found) (6)

$2\ CCl_3^{\bullet} \rightarrow Cl_3CCCl_3$ (k is not found) (7)

$CCl_3^{\bullet} + Fe\ (HEME) \rightarrow Cl_3C\text{-}Fe\ (HEME)$ (k is not found) (8)

$Cl_3C\text{-}Fe(HEME) \rightarrow Cl_2C{=}Fe\ (HEME)$ (k is not found) (9)

[Note: Fe (HEME) is the CYP Fe (HEME) in reaction 8 and 9]

3. CCl_4 activation in mitochondria

CCl_4 metabolism by microsomal enzymes cytochromes P450 has been known for a long time, The CCl_4 metabolism caused by mitochondria was also been found [9,12]. The evidences include the works of the purified mitochondria incubated with CCl_4 and the covalently bond between CCl_4 and mitochondrial DNA is much higher than covalently bond between CCl_4 and nuclear DNA [12]. So several authors have suggested the mitochondrial respiration chain can supply the elections

required for the formation of CCl_4^- and subsequently free radicals; they also suggest the formation of CCl_4^- can happen at the two sites of respiration chain (Scheme.1 and reaction 10,11) [9,12]. By diverting the normal mitochondrial election flow to its own reductive dehalogenation, CCl_4 is acting as an uncoupler of oxidative phosphorylation [12].

NADH → FP_1(4Fe·S) → Q → (2Fe·S) → cyt.*b*(Fe·S) cyt.c_1→ cyt.*c* → cyt.aa_3 → O_2

Scheme1. Scheme representing of the normal mitochondrial electron-transport chain.

$$CCl_4 \xrightarrow{\text{cyt.b(Fe·S)}} CCl_4^- \qquad \text{(k is not found)} \qquad (10)$$

$$CCl_4 \xrightarrow{\text{cyt.aa3}} CCl_4^- \qquad \text{(k is not found)} \qquad (11)$$

4.The activation of CCl_4 in nuclear and the CCl_4works as a carcinogen

CCl_4 has been found a carcinogen in both rats and mice [1-4]. But CCl_4 is found little genotoxic activity in traditional short-term assays and is classified as a non-genotoxic carcinogen [4]. In a Nature paper, CCl_4 has been shown to induce intrachromosomal recombination [5]. Some authors suggested that the free radical species, which can lead the DNA strand breaks, may responsible for their recombinagenic activities [4]. These results are consistent with some earlier research by Gomez *et al.,* in which they show the CCl_4 can also been activated by nuclear preparations, although the activation effects is smaller than the microsomes, but is at the same order of magnitude [13]. In addition, in another paper, Gomez *et al.* showed that the CCl_4 metabolites could form covalent binding with nuclear DNA, protein and lipids [6]. Those results explained the carcinogenic effects CCl_4 to some extent.

5.lipid peroxidation and protein oxidation

It is a well-established fact that lipid peroxidation plays a major role in CCl_4 induced development of fatty liver and cirrhosis [7-9,11-16]. The malondialdehyde (MDA), as a marker of lipid

peroxidation, was found significant increase in (7 fold-14 h value) upon single exposure to CCl_4 vapours [7,14]. The briefly mechanism of lipid peroxidation caused by CCl_4 is showed below [7-9,15-16]

$LH + CCl_3^{\bullet} \rightarrow L^{\bullet} + Cl_3CH$ (k is not found) (12)

$LH + Cl_3C\text{-}OO^{\bullet -} \rightarrow L^{\bullet} + Cl_3C\text{-}OOH$ (k is not found) (13)

$L^{\bullet} + L^{\bullet} \rightarrow 2\ L$ (k is not found) (14)

$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$ ($k \approx 3 \times 10^8\ M^{-1}S^{-1}$) (15)

$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$ ($k \approx 10\text{-}50\ M^{-1}S^{-1}$) (16)

Recently, A significant increase in the Protein Carbonyl (PCo) content of the liver (approximately 2 fold-28 h value) also can be observed in treatment of CCl_4 vapours [14]. Nearly 2 folds increase in liver PCo after single dose of CCl_4 may not appear significant. However, it has been estimated that values as low as 2 nmol of carbonyl per mg protein represents damage to about 10% of total cellular proteins (these are minimal values as the oxidative modification of some amino acid residues in protein does not lead to the formation of carbonyl derivatives) [14]. Extrapolating from this, Sundari PN *et al.* calculated that, in CCl_4 treated rats about 23% of total hepatocellular proteins are damaged. They found significant increase in PCo levels even within an hour of exposure to CCl_4 vapours. This result suggests that the accumulation of oxidised proteins in the liver may be an early indication of CCl_4 induced liver damage. Regarding chronic liver injury, a greater increase in liver PCo was observed (approximately 3 fold) compared to acute liver injury (approximately 2 fold). This result corresponds about 31% of total hepatocellular proteins are damaged[14]. So Sundari *et al.* suggested that, while lipid peroxidation plays a major role in liver injury by CCl_4, oxidative damage to proteins occurs in acute as well as chronic exposure of rats to CCl_4 and may contribute to the pathogenesis of liver injury, Their results also suggest that the accumulation of oxidised proteins in the liver may be an early indication of CCl_4 liver injury [14].

7. Rise of intracellular Ca^{2+} and phospholipase activation caused by CCl_4

Many lines of evidences suggest the secondly mechanism are responsible for the ultimate plasma membrane disruption of the CCl_4 treat cells [9,15-16]. Among them, the cytosol Ca^{2+} concentration increase is particular interested [9,15-16]. A relatively low concentration CCl_4 (0.5 nm) caused a rapid rise in the concentration of cytoplamic free calcium and the extent of Ca^{2+} concentration increase correclates with the degree of live damage [9,15]. Chelation of intracellular Ca^{2+} and block of Ca^{2+} channel can inhibit the appearance of a variety of cellular response to CCl_4 [9,15]. Recently, Hemmings SJ *et al.* isolated mitochondrial, endoplasmic reticular and plasma membrane fractions from the livers of rats given CCl_4 by gavage and found: at 1 h and 24 hours after CCl_4 administration: (i) calcium binding was decreased 65% and 57% in the mitochondrial fraction, 66% and 50% in the endoplasmic reticular fraction and 46% and 71% in the plasma membrane fraction; (ii) calcium uptake was decreased 59% and 55%in the mitochondrial fraction, 46% and 17% in the endoplasmic reticular fraction and 37% and 53% in the plasma membrane fraction. [15]. So all the main Ca^{2+} protect barrier are greatly broken by CCl_4, But exact mechanism of Ca^{2+} activation is still unclear. The Ca^{2+} activation may bring very severe results, one of them is the activation of phospholipase A_2, which cause continual destruction of organnell membranes and inactivation of Ca^{2+} pump and forms a positive feedback to cause more phospholipase A_2 activation. In addition of the lipld peroxodation also promote the increase of phospholipase A_2 activity, which can further cause collapse of membrane structure [9,15]. Another phospholipase, phospholipase C also can cause degradation of membrane phospholipids [16]. Schwerrtz DW *et al.* showed that CCl_4, not its metabolites can activate the phospholipase C directly and cause subsequently phospholipids degradation, so the effects of CCl_4 on the phospholipase C happens very quickly,

can be seen in seconds [16]. Based on their experiments, the authors suggest the ctivate the phospholipase C as a key and early event in the pathogenesis of liver necrosis [16]. The cleave site of phospholipase A_2 and phospholipase C are shown as following figure.

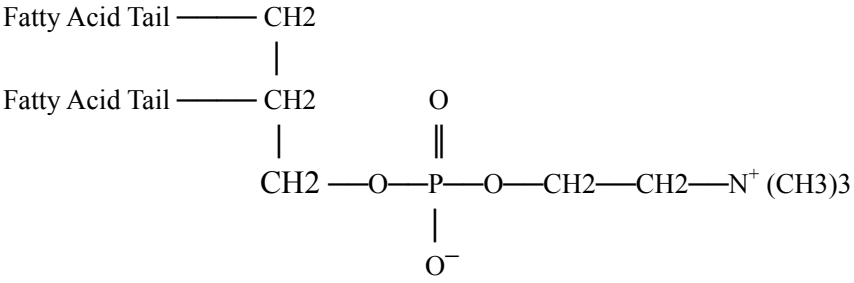

Fig.1.The structure of phospholipid and the cleave site of phospholipase A_2 (site 1) and phospholipase C (site 2) [17]

8. Summary

As both hepatotoxin and carcinogen, Carbon tetrachloride (CCl_4) can induce both acute and chronic liver injury as well as hepatoma. The toxicology of CCl_4 depends on the reductive dehalogenation of CCl_4 catlyzed by cytochromes P450 to a large extend. The trichloromethyl free radical ($CCl_3^{\bullet}$) produced in this process and related reactive species may cause cellular damage by initiating lipid and protein peroxidation, which can cause great damage to cell contents and cause causative effects. Among them the lipid peroxidation plays a particularly important role. But the protein oxidation, DNA oxidation and cascades of secondary mechanisms are also very important in the toxicology of CCl_4. Among the secondary mechanisms, the calcium homeostasis change caused by CCl_4 has a prominent status.

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